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MODIFIED ASSAY PROCEDURE FOR THE ESTIMATION OF SERUM GLUCOSE USING MICROWELL READER

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ABSTRACT

Determination of blood glucose levels is very important to know the physiological condition of the human beings as the hormonal imbalance may cause abnormalities in glucose metabolism. The traditional methods of glucose estimation by colorimetric and titrimetric methods were involved with huge expenditure and time. The modified colorimetric microwell reader method proposed in the present study was performed with small quantities of sample and reagents with the same linearity that was observed in the normal colorimetric analysis. The modified method not only reduces the cost of the test to almost one third of the normal colorimetric method but also provide an opportunity to screen the large number of samples in a short duration of time.

KEY WORDS

Serum Glucose, Colorimetry, Microwell Reader

INTRODUCTION

Glucose is the major carbohydrate found in blood and a chief source of energy in human body. The blood glucose levels are perfectly maintained under the influence of hormones like insulin and glucagon. However, the hormonal imbalance sometimes may result in abnormalities of glucose metabolism and result in diseased condition (1,2,5). Thus, the detection of blood glucose levels can provide a basic understanding of the malfunctioning of the tissues and body. The estimation of glucose levels in blood is being done so far by the colorimetric and titrimetric methods, however these were involved with huge cost and time (3). In the light of its importance, an attempt has been made in the present study to develop a new protocol using microwell reader which can provide an opportunity for the rapid and accurate estimation of glucose. This modified protocol can also reduce the quantity of reagents to be used and it will be a cost effective one.

MATERIALS AND METHODS

The principle involved in the estimation of glucose is that first glucose is oxidized into gluconic acid and hydrogen peroxide. The hydrogen peroxide further reacts with phenol and 4-amino antipyrine by the catalytic action of peroxidase to form a red coloured quinoamine dye complex (4). The reagent used in the present study for the estimation of serum glucose levels was obtained from M/s.Crest Biosystems, Goa, India.

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The protocol for the modified method of glucose estimation is as follows. The glucose reagent supplied along with the kit was aliquoted (300 µl) into 3 different flat bottom microwells of 96 well plate and the wells were marked as blank, test and standard (Table 1). An aliquot of 3 µl of distilled water was added to the well marked as blank, later aliquots of 3 µl of sample and 3 µl of glucose standard (supplied with kit) were dispensed into the test and standard wells respectively. The absorbance of the test was measured at 492 nm against a reagent blank and the concentration of glucose is calculated using standard reference. The results obtained through this procedure was compared with the normal colorimetric method using the same kit.

RESULTS AND DISCUSSION

The results obtained from 20 different patients using the microwell reader method have yielded almost the similar values as that of obtained through colorimetric method with slight variation (Table 2). The data obtained through both the methods was subjected to statistical analysis by calculating the paired t-test of significance to find the variation between both the methods. The results envisaged that the calculated value (P value) was found to be 0.0195 and it is very less when compared to the table value of t-test (1.96) at 5% level indicating the insignificant variation between the colorimetric and microwell reader methods.

Thus the modified method of serum glucose estimation using microwell reader can provide an opportunity to detect the glucose levels very effectively with minimum consumption of reagents with the same linearity as that of the colorimetric method (500 mg/dL). The usage of this method reduces the cost of each test to almost one third of the cost when performed the same with colorimeter in a short time.

Table 1 : Concentration of the reagents used in Colorimetric and Microwell reader methods

Name of the reagent	Colorimetric Method (in µl)	Microwell Reader Method (in µl)
Glucose reagent	3000	900
Sample (for estimation)	10	3
Standard	10	3
Distilled water	10	3

Table 2 : Comparative analysis of serum glucose estimation using Colorimetric and Microwell reader methods

S.NO	Glucose concentration in mg/dL	
	Colorimetric method	Microwell reader method
1	97.2	97.16
2	90.31	90.34
3	101.39	101.49
4	216.93	215.31
5	223.79	221.34
6	196.44	195.76
7	467.80	468.10
8	291.28	291.78
9	79.81	79.15
10	86.90	85.38
11	110.84	111.77
12	134.00	132.80
13	72.80	72.18
14	72.20	72.12
15‘	415.30	408.91
16	611.40	609.80
17	343.00	343.70
18	138.00	134.60
19	85.20	85.10
20	134.00	134.10
Std	95.40	94.80

Each value is the mean of 10 individual observation. The P value (t-test value) is less than 0.05 at 5% significance level

Based on the observations in the present study, the deliberate use of modified protocol is recommended for serum glucose estimation particularly when there is a need for repeated or regular quantification of glucose levels in severe diabetic conditions as it provides an opportunity to screen the multiple number of samples at a time. Considering the precision, reproducibility and cost effectiveness of this method, it is suggested that the modified method could be conveniently adopted for the determination of blood glucose levels.

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